

pET28-Nlact-NCas

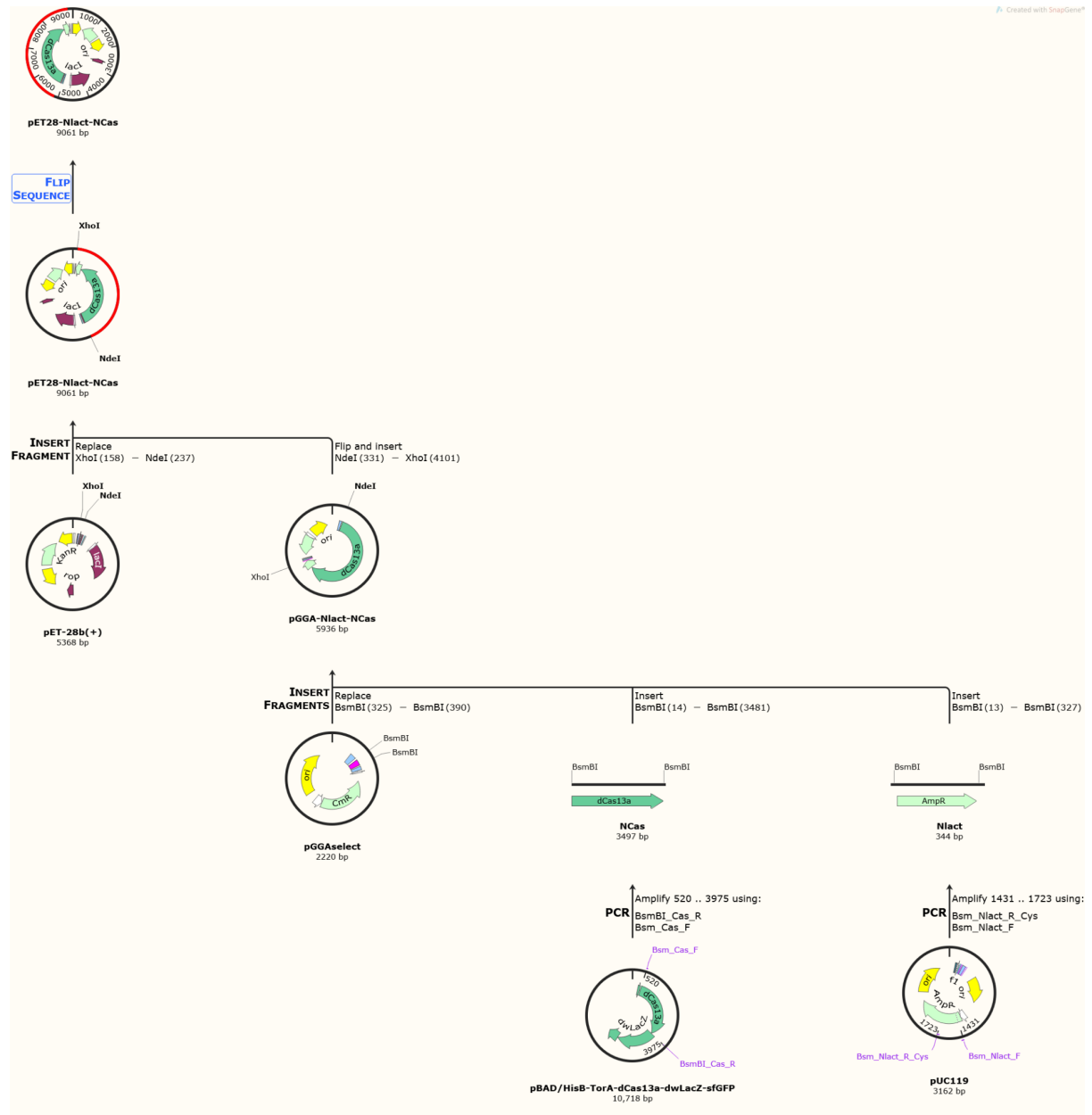


Diagram illustrating the cloning strategy for the pBAD-HisB-TorA-dCas13a-dwLacZ-sfGFP system.

Plasmids and Fragments:

- pET30-Clact-CasC** (8988 bp)
- pET-30a(+)** (5422 bp)
- Clact-CasC** (5918 bp)
- pGGAselect** (2220 bp)
- Clact** (320 bp)
- CasC** (3503 bp)
- pUC119** (3162 bp)
- pBAD/HisB-TorA-dCas13a-dwLacZ-sfGFP** (10,718 bp)

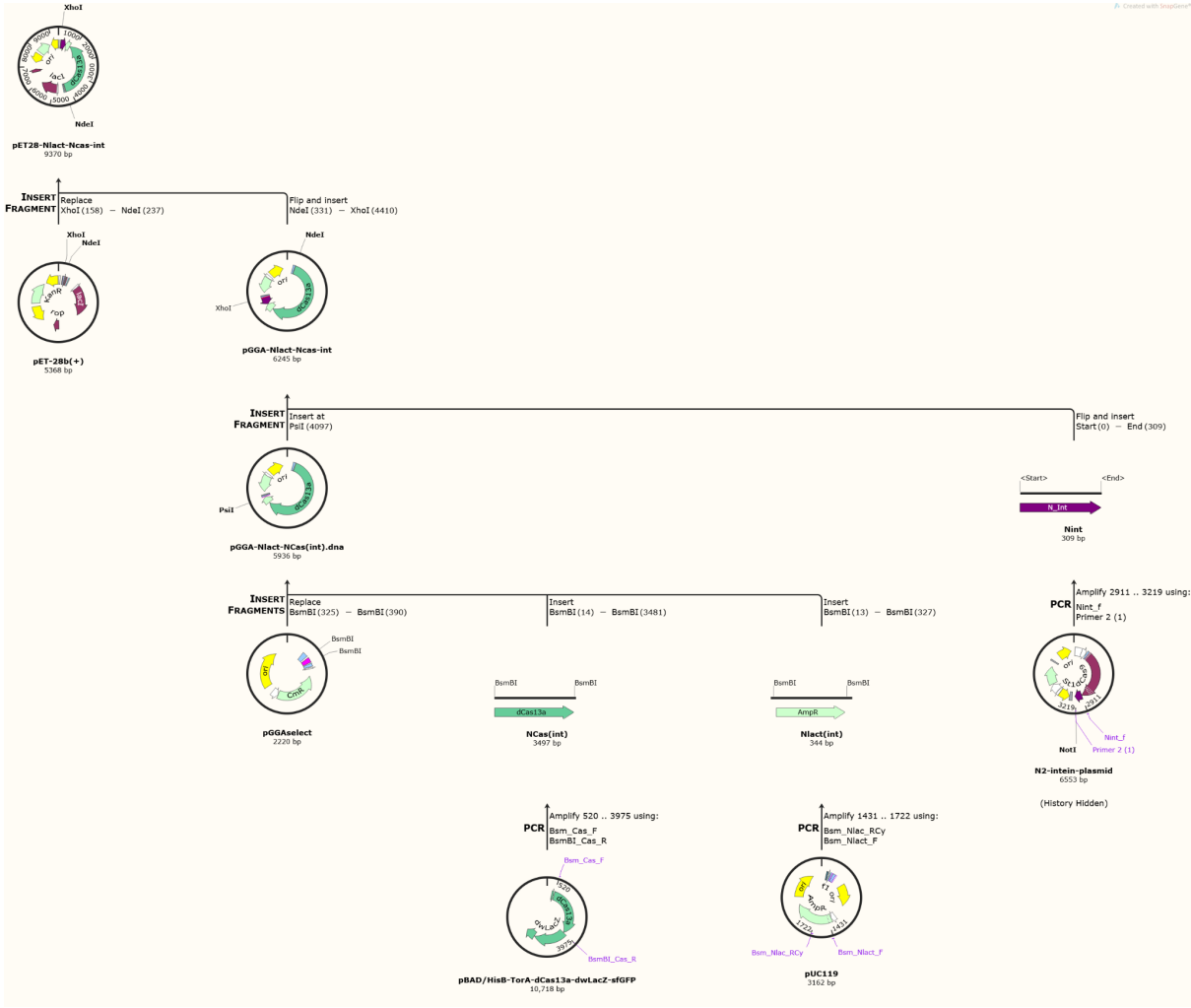
Cloning Strategy:

- Cloning of pET30-Clact-CasC into pET-30a(+):** Replace XhoI (158) - NdeI (346) with the **INSERT FRAGMENT**.
- Cloning of Clact-CasC into pGGAselect:** Replace BsmBI (325) - BsmBI (390) with the **INSERT FRAGMENT**.
- Cloning of Clact and CasC into pUC119 and pBAD/HisB-TorA-dCas13a-dwLacZ-sfGFP:** Insert BsmBI (14) - BsmBI (304) and BsmBI (13) - BsmBI (3486) respectively.

PCR Amplification:

- Clact:** Amplify 1944 .. 2219 using Bsm_Clact_F and Bsm_Clact_R.
- CasC:** Amplify 523 .. 3975 using Bsm_CasC_Fw and Bsm_CasC_R.

pET28-Nlact-Ncas-int



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